ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Clinical Significance of Serum Osteopontin in tumor Progression and Metastasis in Breast Cancer

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Manuscript Info

Manuscript History:

Received: 15 June 2015
Final Accepted: 26 July 2015
Published Online: August 2015

Key words:

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Abstract

Background: Breast cancer is the most common cause of death in females. Early diagnosis, in a high percentage of cases makes it possible to obtain good results in terms of cure and long-term survival but when metastatic lesions are present, the prognosis is very poor. **Aim**: of this study was to assess the possible value of osteopontin (OPN) as a potential biomarker in breast cancer metastasis. **Subject and methods:** 86 breast cancer female patients were included in the study. They were classified into 2 groups: Group 1 (non-metastatic breast cancer patients) include 43 non-metastatic breast cancer female patients, Group 2 (metastatic breast cancer patients) include 43 metastatic breast cancer female patients. Serum osteopontin was determined by ELISA. **Results:** Osteopontin level and CA15-3 level showed high statistically significant difference between group 1 and group 2 ($P < 0.001$) and OPN level showed high statistically significant association with tumor stage ($K = 562.5$ & $P < 0.001$) and tumor grade ($K = 187.4$ &$P < 0.001$). On the other hand there was no statistical significant association between osteopontin level and tumor type ($K= 1.10$ & $P>0.05$). Combined use of OPN and CA15-3 showed 93% sensitivity, 81.4% specificity, 83.3% +ve predictive value, 92.1% -ve predictive value and 87.2% accuracy in predicting tumor distant metastasis. **Conclusion:** The results obtained in this study are valuable for the future application of plasma OPN level as a routine biomarker for the diagnosis and clinical prediction of metastasis, at the time of primary diagnosis of breast cancer.

Key words: osteopontin; breast; cancer; metastasis.

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and the main cause of cancer death in females 'worldwide, accounting for 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths ***(Ferlay, 2010).*** In Egypt breast cancer accounts for 32% among women ***(Ibrahim et al, 2014).*** The majorities of deaths from breast cancer are not due to

the primary tumor itself, but are the result of metastasis to other organs in the body ***(Weigelt et al., 2005)***. Detection of breast cancer metastasis relies on clinical manifestations of the spread to distant organs, biopsies of affected organs, radiological evaluations, imaging methods and serum tumor markers ***(Lacroix,2006)***.Tumor markers that reliably forecast aggressive behavior and poor survival, measured in accessible tissues, such as blood, at the time of metastasis or serially during treatment, would have considerable value in identifying those women who might benefit from early intensive treatments and in suggesting the need to alter therapy if a patient's tumor is not responding ***(Macrì et al., 2009).***

Osteopontin (OPN) is a secreted, integrin-binding glycophosphoprotein, involved in inflammation, wound healing, bone formation and remodeling, as well as atherosclerosis and cancer ***(Cho et al., 2009).*** A wide variety of cell types express OPN, including Osteoblasts, endothelial cells, vascular smooth muscle cells, epithelial cells (such as kidney, breast, and skin), neural cells (neurons, glial cells and Schwann cells) and activated immune cells (such as T-cells, B-cells, macrophages, natural killer (NK) and Kupffer cells) ***(Kunii et al, 2009).***OPN is found as both an immobilized extracellular matrix (ECM) molecule in some tissues(e.g. mineralized) and as a secreted protein in body fluids such as milk, blood, urine, saliva, seminal fluid and bile ***(Rangaswami et al., 2006).*** It signals through different integrin receptors andCD44 variant receptors, through arginine-glycine-aspartate dependent and independent mechanisms, leading to a wide variety of effects ***(Rodrigues et al, 2007).***OPN upon binding with integrins or CD44 regulates breast cancer cell proliferation, migration, invasion and chemotaxis. OPN plays an important role in regulation of tumor progression, angiogenesis and metastasis in breast cancer ***(Chakraborty et al., 2008)***.The ligation of OPN to its receptors stimulates a cascade of signaling pathways which cross talk and foster neoplastic growth in breast cancer ***(Rangaswami et al., 2006).*** So***,*** the aim of this study was to assess the possible value of OPN as a potential biomarker in detecting breast cancer metastasis.

Subject and methods:

This case-control study was conducted in Clinical Pathology and General Surgery Departments in Zagazig University Hospitals during the period from January 2013 to December 2013. After approval from Medical Ethical Committee of Faculty of Medicine, Zagazig University, all participants assigned informed written consent. Eighty six breast cancer female

patients were included in the study. They were classified into 2 groups: Group 1(non-metastatic breast cancer patients) included 43 non-metastatic breast cancer female patients and Group 2(metastatic breast cancer patients) included 43metastatic breast cancer female patients.

Patient inclusion criteria: Newly diagnosed female patients with breast cancer either without metastasis or with documented metastatic breast cancer, who had any site of metastasis outside the loco regional (ipsilateral breast, chest wall, and axilla) area. Preoperative staging carried out with mammography, breast and hepatic ultrasound, chest X-ray, total body bone scan.

Patient exclusion criteria: Patients who had received previous systemic, adjuvant chemotherapy and hormone therapy for metastatic breast cancer. Patients with other diseases that could elevate osteopontin level e.g. (vascular, renal and autoimmune diseases). Patient with other carcinomas that could elevate osteopontin level e.g. (pancreatic adenocarcinoma, head and neck carcinoma brain carcinoma, thyroid carcinoma, hepatocellular carcinoma (HCC), prostatic carcinoma, ovarian carcinoma, breast carcinoma, cancer colon and stomach carcinoma)

All patients were subjected to:

- Clinical evaluation: included detailed history taking and clinical examination, particularly stressed upon: age of diagnosis, menopausal status**,** physical examination to detect palpable mass in the breast, nipple discharge without a palpable mass, asymmetric thickening or nodularity, skin changes and axillary lymph node status, and radiological assessment: Pelvic and abdominal ultrasound, chest X-ray, chest and brain CT and total bone scan to verify and quantify the presence of distant metastases.
- Histological assessment was done including: Histological type was evaluated according to ***Lester et al., (2009)***.Histological grading was evaluated according to Bloom-Richardson breast cancer grading system ***(Edge et al., 2010).***Clinical staging of the breast cancer according to The TNM classification ***(Edge et al., 2010).***
- Laboratory investigation: 3ml of blood were withdrawn from patients and centrifuged serum was frozen and stored at -80˚C until assay. All patients had the following laboratory investigations: Determination of CA15-3 by Cobas Inregra411(Roche, Mannheim, Germany). Determination of osteopontin levels were performed by ELISA (e-Bioscience, San Diego, USA). The sensitivity, intra-assay and inter-assay coefficient of variation were 0.26 ng/ml, 6.7, and 6.1 %, respectively.

Statistical analysis

Analysis of data was performed with Statistical Package for Social Science computer program (SPSS Inc., version 16.0, Chicago, IL). Continuous variables were expressed as mean ± standard deviation and comparison between groups was done using student t-test. The statistical significances of differences in frequencies of variants between the groups were tested using the Chi square test. Mann-Whitney and Krusskall-Wallis tests are used for non parametric not normally distributed data. Mann-Whitney is used for comparing medias of two groups while Krusskall-Wallis test is used for comparing medians of three or more groups. A level of significance was set as $P \leq 0.05$ was considered significant, $P \leq 0.01$ was considered highly significant and $P > 0.05$ was considered not significant.

RESULTS

The Demographic characteristics of the studied groups are shown in **Table 1**. There were no significant differences among studied groups regarding age, and menopausal state (P-value >0.05).

According to the histological characteristics of tumor among two studied groups there is high statistically significant difference in tumor stage (p-value<0.001) between group 1 and group 2. Grade II is the most common tumor grade among two studied groups and there is no statistically significant difference in tumor grade between group 1and group 2 (p-value >0.05). Invasive duct carcinoma is the most common tumor type among two studied groups and there is no statistically significant difference in tumor type between group 1 and group 2 (p-value >0.05) **Table2.**

Osteopontin level and CA15-3 level show high statistically significant difference between group 1 and group 2 (P-value <0.001) as shown in **table (3)**.

The sites of distant metastasis were bone (41.9%), liver (20.9%), lung (9.3%), brain (2.3%), bone and liver (7%), bone and lung (11.6%) and bone and brain (7%) and there is no statistically significant difference in osteopontin level between sites of distant metastasis (K = 4.73, P-value >0.05) as shown in **table (4)**.

OPN level shows high statistically significant association with tumor stage (K = 562.5 & P-value < 0.001) and tumor grade (K = 187.4 & P-value <0.001) between the two studied groups. On the other hand there is no statistical significant association between osteopontin level and tumor type (K= 1.10 & P-value >0.05). According to the menopausal state there is high statistically significant association between osteopontin and menopausal state (Z =249.7 & p-value <0.001) in group 1 (Z = 177.1, p-value <0.001) in group 2 **table (5).**

ROC curve analysis for CA15-3 levels in detection of breast cancer metastasis shows that at cutoff value 26.8 u/ml, CA 15-3 can detect 34 0ut of 43 metastatic breast cancer patients while 6 out of 43 non metastatic breast cancer patient mis-diagnosed as having metastatic breast cancer.CA15-3 has 79.1% sensitivity is , 88.1% specificity 87.1%,+ve predictive value, 80.4% -ve predictive value and83.5 % accuracy in detection of tumor distant metastasis, While ROC curve analysis for OPN levels shows that the best cutoff value of OPN is 36ng/ml which can detect 38 out of 43 metastatic breast cancer patients while 8 cases out of 43 non metastatic breast cancer patient mis-diagnosed as having metastatic breast cancer. OPN has 88.4% sensitivity, 81.4% specificity, 87.5%+ve predictive value, 87.5% -ve predictive value and 84.8% accuracy in predicting tumor distant metastasis. However, ROC curve combined for OPN (ng/ml) and CA15-3 (u/ml) shows sensitivity 93 %, specificity is 81.4%, +ve predictive value 83.3%,-ve predictive value 92.1% and accuracy 87.2% in predicting tumor distant metastasis as shown in **table(6) , Fig 1.**

Table (1): Demographic characteristics of the studied groups.

	Group(1) **n= (43)**	**Group(2)** **n= (43)**	**Test of significance**	**P-value**	**Sig**
Age in years Mean ± (SD)	48.62±9.35	51.95±11.89	t test=1.44	>0.05	NS
Menopausal state					
Premenopausal N (%)	21(48.8%)	18(41.9%)	X^2= 0.42	>0.05	NS
Postmenopausal N(%)	22 (51.2%)	25(58.1%)			

Table (2): Tumor stage, Tumor grade and Tumor type among two studied groups).

	Group1(N=43)		Group2(N=43)		X^2	P-value	Sig
	No	%	No	%			
Tumor stage							
Stage II	28	65.1	0	0.0	87.6	<0.001	HS
Stage III	15	34.9	0	0.0			
Stage IV	0	0.0	43	100.0			
Tumor grade							
Grade I	1	2.3	0	0.0	5.4	>0.05	NS
Grade II	28	65.1	20	46.4			
Grade III	14	32.6	23	53.6			
Tumor type							
Invasive duct	38	88.4	42	97.7	4.2	>0.05	NS
lobular	4	9.3	0	0.0			
mucinous	1	2.3	1	2.3			

Table (3): Osteopontin level (ng/ml) and CA15-3 (u/ml) among two studied groups.

	Group(1) N (43)	Group(2) N (43)	Mann Whitney	P-value	Sig
Osteopontin (ng/ml)					
Median	9.5	101.3	108	<0.001	HS
Range	2.35-95	19.5-180			
CA15-3*					
Median	17.9	52	288.0	<0.001	HS
Range	5.80-57.8	5.54-1064.5			

Normal range of CA15.3= 0-25 u/ml

Table (4): Site of distant metastasis within all metastatic breast cancer patients (group 2) and osteopontin (ng/ml) levels in each site.

Site of distant metastasis	No	%	Osteopontin Median	Range	K	P	Sig.
Bone	18	41.9	105	22-180	4.73	>0.05	NS
Liver	9	20.9	106.3	79-140			
Lung	4	9.3	76.3	30.5-105			
Brain	1	2.3	100	----			
Bone and liver	3	7	90	25-130			
Bone and lung	5	11.6	105	67.5-145			
Bone and brain	3	7	137.5	95-180			

Table (5): **OPN (ng/ml) association with tumor stage, tumor grade, tumor type, and menopausal state.**

OPN	Median	Range	K	P-value	Sig
Tumor stage					
Stage II	10.5	2.35-65			
Stage III	77.5	19-95	562.5	<0.001	HS
Stage IV	100	29.5-180			
Tumor grade					
Grade I	8.5	--			
Grade II	65	2.35-140	187.4	<0.001	HS
Grade III	87.5	8.50-180			
Tumor type					
IDC	65	2.35-180			
ILC	95	11.5-105	1.10	>0.05	NS
Mucinous	105	11.5-137.5			
Menopausal State	**Median**	**Range**	**MW**	**P-value**	**Sig**
Premenopausal					
Group I	10.8	2.35-90	249.7		
Group II	97.5	22.5-180		<0.001	HS
Postmenopausal					
Group I	9.5	2.35-95	177.1	<0.001	HS
Group II	103.8	19.5-180			

Table (6): Sensitivity, specificity, +ve predictive value, -ve predictive value and accuracy of combined OPN and CA15-3 for detection of breast cancer metastasis.

	Sensitivity	Specificity	+VE predictive	-VE predictive	Accuracy
CA15-3 Cut off 26.8 u/ml	79.1%	88.1%	87.1%	80.4%	83.5%
OPN Cut off 36 ng/ml	88.4%	81.4%	82.6%	87.5%	84.8%
Combined OPN(ng/ml) and CA15-3(u/ml)	93%	81.4%	83.3%	92.1%	87.2%

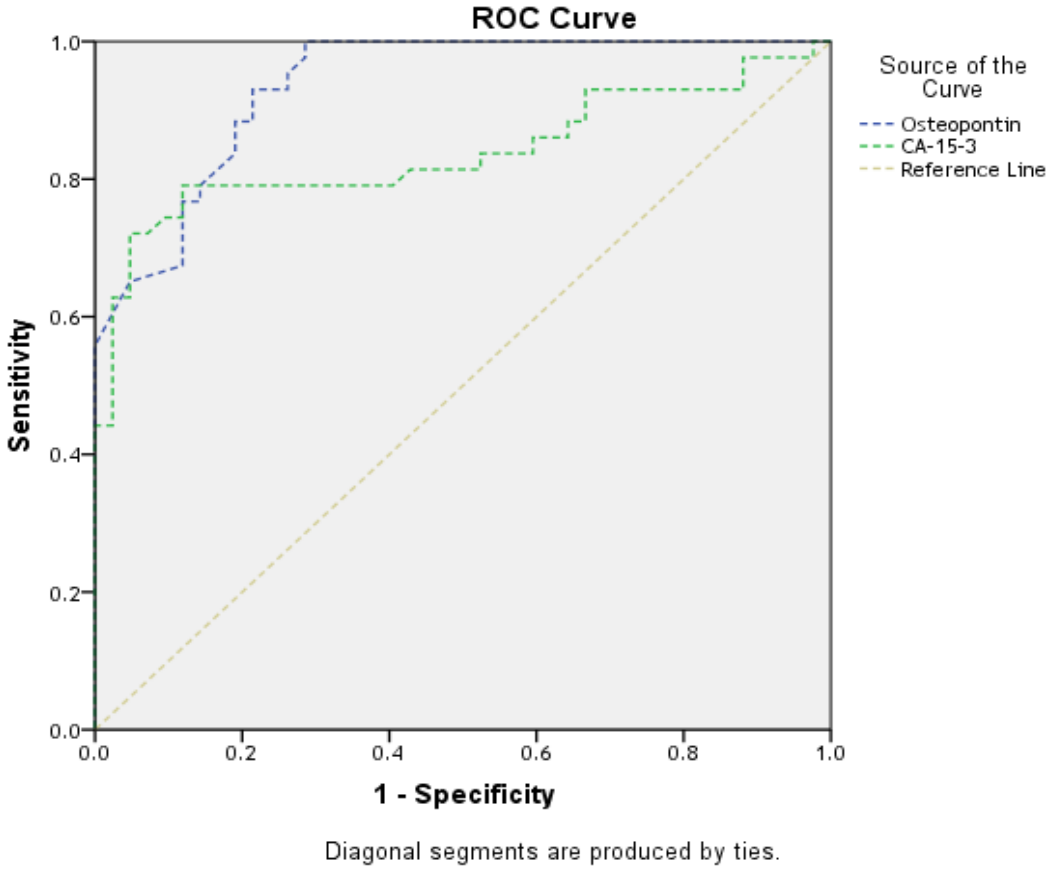

Figure (1): ROC curve analysis for OPN and CA15-3 levels indetection of breast cancer metastasis. The area under the curve (AUC) for OPN was = 0.936 The area under the curve (AUC) for CA15-3 was= 0.838

Discussion:

In this study there was very high statistically significant difference in osteopontin level between the studied groups (p-value <0.001). This result coincide with study done by **Bramwell et al.,(2006)**who showed that enhanced expression of OPN has been found in plasma and tumors of metastatic breast cancer suggesting that OPN may be considered as a prognostic marker. Categorical meta-analysis done by **Weber et al.,(2011**) on female patient with breast cancer showed that Osteopontin level in primary tumors vs. metastases was statistically significant. This result also consistent with a study carried out by **Macrì et al., (2009) who** reported that in the 22 patients who did not have metastatic bone lesions (group A), the preoperative serum levels of osteopontin documented a very low concentration of the glycoprotein, whereas in the 4 patients

with bone metastases (group B), osteopontin measurement showed higher serum levels. In the present study there was very high statistically significant difference between group 1and group 2 as regard CA15-3 level, (p-value <0.001), Similar findings to the present study were reported by **Sandri et al., (2012)**. As regard sites of distant metastasis , they were bone(41.9%), liver (20.9%), lung (9.3%), brain (2.3%), bone and liver (7%), bone and lung (11.6%) and bone and brain (7%) and there was no statistically significant difference in osteopontin level between sites of distant metastasis (P-value >0.05) and this supported by **Berman et al., (2013).** In the present study There was high statistically significant association between osteopontin level and tumor grade (P <0.001) and tumor stage (P<0.001),but there is no statistically significant association between osteopontin and tumor type (P >0.05).This results were in agreement with **Rudland et al.,(2002)**. Similar findings to the present study were also reported by **Weber et al., (2010**) who found that There was statistical significant association between osteopontin and tumor size (p<0.001) ,tumor grade (p <0.001), lymph node metastasis (p < 0.001) ,distant metastasis (p < 0.001) in studies made on breast cancer. Our results were inconsistent with **Rodrigues et al, (2009)** who examined invasive breast tumors and showed that no statistical significant association exists between osteopontin expression and angiogenesis or major clinicopathological parameters such as nodal status (p=0.107) and tumor size (p=0.572), tumor type (p=0.304), tumor grade (p=0.941) but results of tumor type in agreement with present study, this due to difference in sample type and sample size. In our study There was high statistically significant association between osteopontin and menopausal state (p<0.001) in group1, (p<0.001) in group2 and this was in agreement with **Cho et al., (2013**). The sensitivity and specificity of OPN has been shown to vary with the different cutoff values used. According to our results, the sensitivity and specificity of OPN for selective detection of metastatic breast cancer over the non-metastatic group were 36ng/ml and the cut off of CA15.3 that differentiate the metastatic and non-metastatic breast cancer is 26.8u/ml which were comparable to **Mohamed zadeh et al.,(2010).**The present study revealed that combined OPN (ng/ml) and CA15-3 (u/ml) sensitivity is 93 %, specificity is 81.4%, +ve predictive value 83.3%,-ve predictive value 92.1% and accuracy 87.2% in predicting tumor distant metastasis. This mean increase sensitivity, +ve predictive value, -ve predictive value and accuracy when both markers used combined.

Conclusion

The results obtained in this study are valuable for the future application of plasma OPN level as a routine biomarker for the diagnosis and clinical prediction of metastasis, at the time of primary diagnosis of breast cancer.

Conflict of interest

The authors have no conflict of interest to declare.

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